Exposing young guinea pigs to sidestream tobacco smoke decreases rapidly adapting receptor responsiveness

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Bonham, A. C., C. T. Kappagoda, K. S. Kott, and J. P. Joad. Exposing young guinea pigs to sidestream tobacco smoke decreases rapidly adapting receptor responsiveness. *J. Appl. Physiol.* 78(4): 1412–1420, 1995.—We exposed 21 young guinea pigs to 5 wk of either sidestream tobacco smoke (SS) or filtered air (FA). The exposure started on *day 8* of life and ended at 41–45 days of life. The animals were then anesthetized, and lung rapidly adapting receptor (RAR) and slowly adapting receptor (SAR) activities and peak tracheal pressure (TP) were examined in response to mainstream smoke. SS exposure did not alter baseline RAR activity. Low-nicotine smoke increased RAR activity in the FA but not in the SS group. High-nicotine smoke increased RAR activity in both groups but more so in the FA than in the SS group. Baseline TP was lower in the SS group. Both low- and high-nicotine smoke increased TP but more so in the FA than in the SS group. The increase in RAR activity preceded the increase in TP. SS exposure increased baseline SAR activity but did not affect the variable responses of SARs to low- and high-nicotine smoke. We suggest that exposing guinea pigs to SS during development diminishes the responsiveness of RARs to acute inhalation of mainstream smoke.

environmental tobacco smoke; nicotine; airways; vagus

IT IS WELL DOCUMENTED that children living in homes where they are exposed to environmental tobacco smoke (ETS) have increased respiratory problems. For example, ETS-exposed children have more bed disability days, as evidenced by a survey of 37,000 households (3); increased cough with colds (2, 6, 7); increased wheeze and sputum production (6); and increased risk of respiratory illness-related hospitalizations (7). Pulmonary function evaluations have documented that children raised with ETS have decreased forced expiratory volume in 1 s (22, 31), forced expiratory volume in 1 s/forced vital capacity (27), and maximal midexpiratory flow rate (20, 22), suggesting that their airways are partially obstructed. Children exposed to ETS also exhibit increased airway reactivity (9, 20, 36), an increased rate of asthma (20, 33), and an earlier (1st year of life) onset of asthma. Furthermore, for children with asthma, ETS exposure is associated with more severe asthma and greater airway reactivity (20, 33). Collectively, these studies suggest that children exposed to ETS have increased respiratory symptoms, airway obstruction, increased airway reactivity, and a higher incidence and severity of asthma.

These respiratory symptoms of cough and airway obstruction may result from a chronic stimulation of C-fiber receptors and the rapidly adapting (irritant) receptors (RARs) in the lungs and airways. Stimulation of these receptors elicits airway defense mechanisms that include cough, bronchoconstriction, and increased mucus secretion (4). Moreover, electrophysiological studies have shown that the RARs and the pulmonary C-fiber receptors are exquisitely sensitive to the inhalation of mainstream cigarette smoke (17, 18, 23, 26). What is not known is the effect of chronic exposure to ETS on the responsiveness of these receptors. It is possible that the airway defense mechanisms elicited by stimulation of these receptors, although still apparent, are not as vigorous in those children who have experienced long-term exposure to ETS during their development. There is precedent for a diminished responsiveness of these receptors after repeated exposure to mainstream tobacco smoke. First, the airway irritation and cough produced by the first puff of a cigarette by the naive smoker subsides with repeated smoking. Second, Swanny et al. (30) have shown that chronic exposure to mainstream tobacco smoke for 4–8 wk attenuates the reflex breathing responses to acute inhalation of tobacco smoke in rats, implying a decreased responsiveness of the vagal sensory receptors. It may also be the case that, in children exposed to ETS, the RARs and C-fiber receptors in the lungs and airways become less responsive and, consequently, elicit diminished defensive responses, leaving the lung more vulnerable to other noxious agents. This may explain, at least in part, the increased frequency and severity of respiratory problems in those children.

In the present study, we focused on the potential contribution of the RARs. We exposed developing guinea pigs to either sidestream smoke or filtered air for ~5 wk and then examined the responsiveness of their RARs. We hypothesized that exposing developing guinea pigs to sidestream smoke would diminish the responsiveness of the RARs to acute stimulation. For the acute stimulus, we used mainstream tobacco smoke from low and high nicotine-containing cigarettes because it is a potent stimulus for the RARs in multiple species including the guinea pig (1, 17, 18, 23, 26). We also examined the activity of slowly adapting pulmonary receptors (SARs) under the corresponding conditions to determine whether exposure to sidestream smoke would also alter the responsiveness of another vagal sensory receptor to acute stimulation by mainstream smoke.

METHODS

Chronic exposure to sidestream tobacco smoke. Male Dunkin-Hartley guinea pigs were randomly assigned to a group exposed to either sidestream smoke (n = 11) or filtered air (n = 10) for 6 h/day, 5 days/wk, from 8 to 41–45 days of life. Sidestream smoke is a surrogate for ETS, differing only in

mainstream response vs. sidestream response?

that it does not contain expired mainstream tobacco smoke. The guinea pigs were housed in polycarbonate cages (69 × 69-cm cross-sectional area) with wire lids and autoclaved wood carvings for bedding. They were fed guinea pig chow and water ad libitum, including during the exposures. The exposure system and monitoring methods have been previously described (32). Briefly, sidestream smoke was generated by a modified ADL/II smoke exposure system (Little, Cambridge, MA) from conditioned 1R4F cigarettes from the University of Kentucky Tobacco and Health Research Institute. Two cigarettes at a time were smoked under Federal Trade Commission conditions in a staggered fashion at a rate of 1 puff (35 ml, 2-s duration) per minute. The mainstream smoke was collected on a filter and discarded. The sidestream smoke was diluted in a 1:10 ratio with filtered air in a mixing chamber and then passed into the stainless steel and glass Hinners-type exposure chamber 0.44 m^3 in size. The exposure chamber was characterized by a relative humidity of 46 ± 8%, temperature of 23 ± 0.9°C, respirable suspended particulate concentration of 1.02 ± 0.07 mg/m^3, carbon monoxide concentration of 6.16 ± 0.62 ppm, and nicotine concentration of 345 ± 99 $\mu g/m^3$. Relative humidity and temperature were sampled continuously. Nicotine was sampled for 15 min twice during each 6-h exposure period. The respirable suspended particulate concentration was sampled using the piezobalance technique for 30 min out of every hour.

Experimental procedures. During the data-acquisition period, the investigators were blinded as to whether the guinea pigs had been exposed to filtered air or sidestream smoke. Each guinea pig was anesthetized with an intraperitoneal injection of 1.7 mg/kg of urethan and then given supplemental doses of pentobarbital sodium (4 mg/kg iv) about every hour, as needed. Catheters were introduced into the jugular vein for administering fluids and drugs and into the carotid artery for monitoring arterial blood pressure and withdrawing blood samples for blood gases. The trachea was cannulated below the larynx, and a catheter was connected to a side port of the endotracheal tube to monitor intratracheal pressure. The guinea pigs were prepared with a bilateral pneumothorax by incisions made in the chest wall and were mechanically ventilated with oxygen-enriched air with a tidal volume of 8 ml/kg at a rate of 33 ± 5 breaths/min. The expiratory line of the ventilator was placed under 2 cm of water. Arterial blood gases and pH were maintained within normal limits by adjusting the ventilator rate and by infusing sodium bicarbonate. The animals were paralyzed with gallamine (1 mg/kg) every hour, as needed. During neuromuscular blockade, the adequacy of anesthesia was continuously assessed by monitoring the animal for spontaneous fluctuations in arterial blood pressure. About every hour, the animals were allowed to recover from the gallamine, at which time we assessed the adequacy of anesthesia by testing for the absence of an increase in systemic arterial pressure heart rate that occurred in response to a paw pinch. Body temperature was monitored with a thermistor and maintained with a servo-controlled water blanket.

For recording RAR or SAR afferent activity, the left cervical vagus nerve was transected below the nodose ganglion, and the distal end was placed on a dissecting platform in a pool of mineral oil. Afferent nerve activity was recorded from nerve bundles dissected away from the transected vagus nerve. A nerve bundle containing an RAR or SAR afferent fiber was split down so that the fiber was the only active fiber discernible or whose signal-to-noise ratio was sufficient to differentiate its activity from the noise by use of a window discriminator. Identification of RARs was based on their rapid adaptation to a fast-rising, then maintained, hyperinflation (~2–3 tidal volumes) (16, 34). Identification of SARs was based on their high-frequency burst with each lung inflation (16) and no or very little adaptation to maintained lung inflation.

For inhalation of mainstream cigarette smoke, the main stem of a Y connector was attached in series to the inlet of the ventilator. One arm of the Y connector was attached to the oxygen-enriched room air source; the second arm was attached to a cigarette holder and was always clamped off during normal ventilation. For safety reasons, 3 min before the cigarette was lighted, the oxygen was turned off and the animal was ventilated with room air. A lighted cigarette was connected to the holder attached to one arm of the Y connector, and the clamp was moved to the second arm (connected to the air) so that during each ventilatory cycle air was drawn through the cigarette into the inlet of the ventilator. Each breath of cigarette smoke delivered by the ventilator was equal to the tidal volume of the ventilator. In preliminary experiments, an unlighted cigarette was attached to one arm of the Y connector to establish that connection of the cigarette alone produced no changes in peak or end-expiratory airway pressure. The cigarettes used for the mainstream inhalation were developed by the University of Kentucky Tobacco and Health Research Institute. The low-nicotine cigarettes (series 4A1) contained 0.17 mg of nicotine and 31.4 mg of tar per cigarette. The high-nicotine cigarettes (series 1A4) contained 2.2 mg of nicotine and 29.5 mg of tar per cigarette. The nicotine content of both the low- and high-nicotine cigarettes was comparable to the content of commercially available brands, which ranges from 0.1 mg/cigarette (e.g., Carlton 100) to 2.1 mg/cigarette (English Ovals King Size) (8).

Once an RAR or SAR fiber was identified, the afferent activity and peak tracheal pressure were recorded during an initial control period of 60 s, an experimental period of 60 s that began after the third breath of smoke inhalation, and a second control (recovery) period of 60 s. This second 60-s control period was taken from 2 to 10 min after inhalation of low-nicotine cigarette smoke, although RAR activity generally returned toward the control value within 2 min. After the second control period, high-nicotine smoke was delivered and the afferent activity and peak tracheal pressure were recorded for a second experimental period of 60 s; this was followed by a final control period of 60 s.

In pilot studies in guinea pigs, we determined that delivery of three tidal volumes of low-nicotine cigarette smoke every 2–5 min does not result in tachyphylaxis. Ravi et al. (23) and Zhang and Bonham (38) have also shown in the rabbit that there is a nicotine concentration-dependent increase in RAR activity regardless of whether smoke from high- or low-nicotine cigarettes is inhaled first.

Data analysis. The afferent activity was recorded in 3-s bins. To determine differences in RAR activity between the sidestream smoke- and filtered air-exposed groups, we used an unpaired *t*-test. Such comparisons of afferent activity were

TABLE 1. *Weight and blood gases for animals in filtered air- and sidestream smoke-exposed groups*

	Filtered Air	Sidestream Smoke
Weight, g	449±52	459±44
Po_2, Torr	281±119	291±121
Pco_2, Torr	36±7	37±5
pH	7.39±0.06	7.38±0.05

Values are means ± SE; n = 10 animals in filtered air-exposed group and 11 animals in sidestream smoke-exposed group

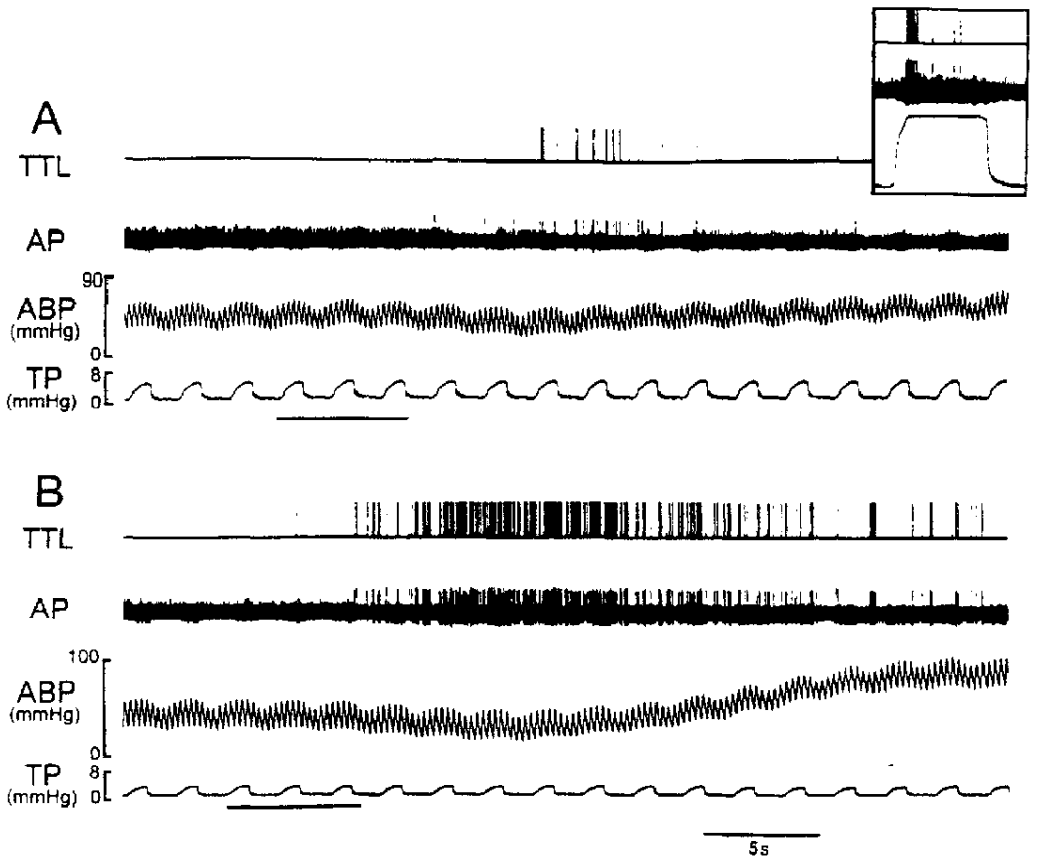

FIG. 1. Effect of mainstream smoke on activity of a rapidly adapting receptor (RAR) from a filtered air-exposed guinea pig. Three breaths of low-nicotine mainstream cigarette smoke produced an increase in RAR activity that began with 1st breath after smoke was inhaled (*A*). Effect was greater and lasted longer after inhalation of high-nicotine smoke (*B*). *Inset*, rapid adaptation to lung hyperinflation. TTL, transistor-transistor pulses from output of a window discriminator; AP, action potential; ABP, arterial blood pressure; TP, tracheal pressure. Bar, smoke delivery.

made among the baseline values (the initial control period of twenty 3-s bins), an experimental period after smoke was inhaled (which was analyzed over the first 10 bins), and a second control (recovery) period of 20 bins. Comparisons were made for both low- and high-nicotine cigarettes. To determine whether the changes in afferent activity after mainstream smoke inhalation were statistically significant within either the sidestream smoke- or filtered air-exposed groups, we used analysis of variance (ANOVA) for repeated measures followed by Scheffé's F tests when appropriate. For individual afferents, a response was designated if there was a 15% change from baseline activity.

To determine differences in peak tracheal pressure between the sidestream smoke- and filtered air-exposed groups, we used an unpaired t-test. Such comparisons of peak tracheal pressure were made between the baseline values (the initial control period of 20 breaths) and an experimental period of 20 breaths after smoke was inhaled. Comparisons were made for both low- and high-nicotine cigarettes. To determine whether the changes in peak tracheal pressure after mainstream smoke inhalation were statistically significant within either the sidestream smoke- or filtered air-exposed groups, we used a paired t-test.

Baseline and peak changes in arterial blood pressure evoked by mainstream cigarette smoke were also compared for the sidestream smoke- and filtered air-exposed guinea pigs by using the unpaired t-test. The paired t-test was used to determine whether the increases in arterial blood pressure were statistically significant after mainstream smoke inhalation within either the sidestream smoke- or filtered air-exposed groups.

The data are reported as means ± SE. Significance levels for all analyses were set at $P < 0.05$.

RESULTS

Eleven RARs and 21 SARs were recorded in 11 guinea pigs exposed to sidestream smoke. Fourteen RARs and 16 SARs were recorded in 10 guinea pigs exposed to filtered air. The weights and arterial blood gases were not different in the two groups (Table 1).

Responses of RARs. Five weeks of exposure of the developing guinea pig to sidestream smoke diminished the responsiveness of the RARs to inhalation of mainstream smoke from low- and high-nicotine cigarettes. An example of the response of an RAR recorded from a guinea pig exposed to filtered air is shown in Fig. 1.

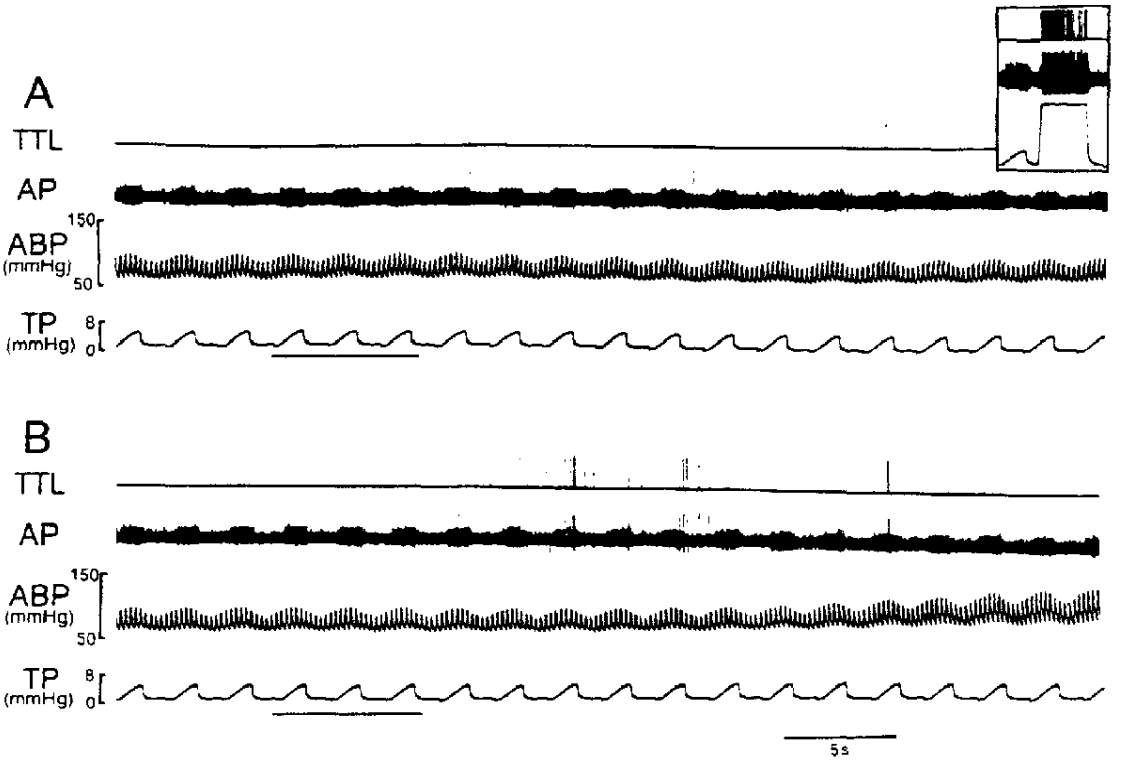

FIG. 2. Effect of mainstream smoke on activity of an RAR from a sidestream smoke-exposed guinea pig. Three breaths of low-nicotine mainstream cigarette smoke produced a modest increase in activity that began with 1st breath after smoke was inhaled (*A*). Effect was greater and lasted longer after inhalation of high-nicotine smoke (*B*). *Inset*, rapid adaptation to lung hyperinflation. Bar, smoke delivery.

The receptor was quiescent under baseline conditions but was excited in a dose-dependent manner by three breaths of mainstream smoke from a low- and a high-nicotine-containing cigarette.

An example of the response of an RAR recorded from a guinea pig exposed to sidestream smoke is shown in Fig. 2. The receptor, also quiescent under baseline conditions, was excited in a dose-dependent manner by three breaths of mainstream smoke from a low- and a high-nicotine-containing cigarette but to a lesser degree than was the RAR from the filtered air-exposed animal.

The grouped data for the 11 RARs from animals exposed to sidestream smoke and for the 14 RARs from animals exposed to filtered air are shown in Fig. 3. The baseline RAR activity was not different between the two groups (4.19 ± 1.62 vs. 3.21 ± 2.07 spikes/bin in the filtered air- and sidestream smoke-exposed groups, respectively; $P = 0.35$ by unpaired t-test). Inhalation of mainstream smoke from low-nicotine cigarettes increased the activity (measured over 10 bins) of the RARs from filtered air-exposed animals from 4.19 ± 1.62 to 32.1 ± 9.7 spikes/bin ($P < 0.002$ by ANOVA; $P < 0.05$ by Scheffé's test). However, low-nicotine cigarette smoke did not significantly increase the activity of RARs recorded in sidestream smoke-exposed animals; the RAR activity was 3.21 ± 2.07 spikes/bin during the control period vs. 8.66 ± 4.67 spikes/bin during 10 bins after smoke ($P = 0.17$ by ANOVA). The activity of the RARs in the filtered air-exposed guinea pigs was significantly greater after low-nicotine smoke inhalation than that of the RARs in the sidestream smoke-exposed group ($P < 0.03$ by unpaired t-test). The RAR activity in both groups returned to baseline values after 20 bins (6.02 ± 1.77 spikes/bin for the filtered air-exposed group and 3.74 ± 2.77 spikes/bin for the sidestream smoke-exposed group). These values were not different from each other ($P = 0.24$ by unpaired t-test) and served as the control values for the response to high-nicotine smoke. Inhalation of mainstream smoke from high-nicotine cigarettes increased the activity (measured over 10 bins) of the RARs from filtered air-exposed animals from 6.02 ± 1.77 to 42.7 ± 6.7 spikes/bin ($P = 0.04$ by ANOVA; $P < 0.05$ by Scheffé's test). Whereas high-nicotine cigarette smoke significantly increased the activity of the RARs from the sidestream smoke-exposed group from 3.74 ± 2.77 to 22.14 ± 3.17 spikes/bin ($P = 0.04$ by ANOVA; $P < 0.05$ by Scheffé's test), the increase was significantly smaller compared with increase in RAR activity from the filtered air-exposed group ($P = 0.037$ by unpaired t-test).

Responses of peak tracheal pressure and arterial blood pressure. The baseline peak tracheal pressure was 19% lower in the sidestream smoke-exposed

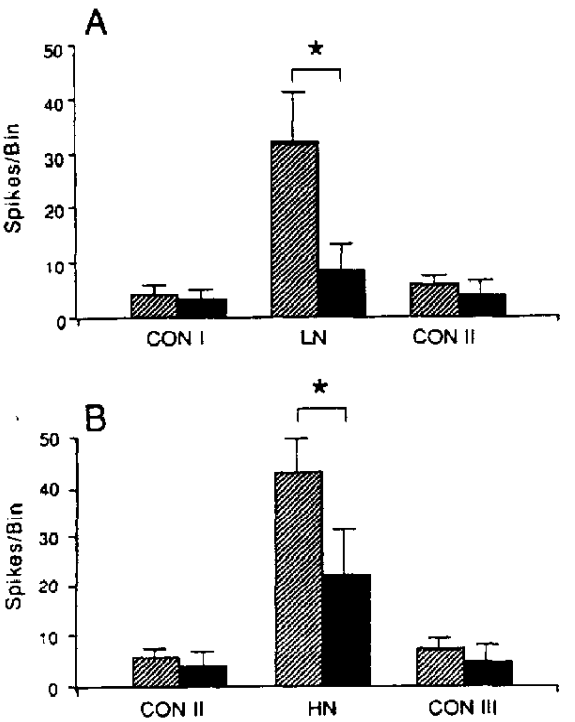

FIG. 3. Summary of mainstream smoke effects on RARs from filtered air- (hatched bars) and sidestream smoke-exposed (solid bars) animals. Values are means ± SE. Baseline activity of 14 RARs recorded in filtered air-exposed guinea pigs and 11 RARs recorded in sidestream smoke-exposed guinea pigs was not different, but activity after low-nicotine (LN; *A*) smoke was greater in filtered air-exposed group (* $P < 0.03$ by unpaired *t*-test). LN smoke inhalation significantly increased activity of RARs in filtered air- but not in sidestream smoke-exposed group. RAR activity in both groups recovered. High-nicotine (HN; *B*) mainstream smoke increased activity of RARs in both groups, but RAR activity in filtered air-exposed group was significantly higher than that in sidestream smoke-exposed group (* $P = 0.04$ by unpaired *t*-test). CON I, CON II, and CON III, 1st, 2nd, and 3rd control periods, respectively.

guinea pigs compared with the filtered air-exposed group (4.84 ± 0.39 vs. 5.99 ± 0.47 mmHg in the sidestream smoke- and filtered air-exposed groups, respectively; $P < 0.03$ by unpaired *t*-test). After low-nicotine smoke, peak tracheal pressure increased in the filtered air-exposed group from 5.99 ± 0.47 to 7.44 ± 1.11 mmHg ($P < 0.05$ by paired *t*-test) and in the sidestream smoke-exposed group from 4.84 ± 0.39 to 5.09 ± 0.46 mmHg ($P < 0.05$ by paired *t*-test). After high-nicotine smoke, peak tracheal pressure increased in the filtered air-exposed group from 6.61 ± 0.49 to 7.99 ± 0.86 mmHg ($P < 0.05$ by paired *t*-test) and in the sidestream smoke-exposed group from 5.31 ± 0.47 to 6.27 ± 0.87 mmHg ($P < 0.05$ by paired *t*-test). To compare changes in peak tracheal pressure between the filtered air- and sidestream smoke-exposed groups after smoke inhalation, we used the percent increase over baseline values. In the sidestream smoke-exposed animals, the percent increase in peak tracheal pressure after low-nicotine smoke was significantly lower than in the filtered air-exposed group (4.74 ± 1.65 vs. 21.0 ± 1.8% in the sidestream smoke- and filtered air-exposed groups, respectively; $P = 0.03$ by unpaired *t*-test). However, the responses of the peak tracheal pressure to high-nicotine cigarette smoke in both groups approached parity, with a 15.5 ± 5.6% increase in the sidestream smoke-exposed group vs. a 19.8 ± 7.2% increase in the filtered air-exposed group ($P = 0.30$ by unpaired *t*-test).

Resting mean arterial blood pressure averaged 51 ± 4 mmHg in the filtered air-exposed group and 50 ± 1 mmHg in the sidestream smoke-exposed group. Inhalation of low-nicotine mainstream smoke had no effect on arterial blood pressure. However, high-nicotine smoke increased it similarly in both groups, by 12 ± 12 mmHg in the sidestream smoke-exposed animals and by 16 ± 12 mmHg in the filtered air-exposed animals. In both instances, the peak increases were not different ($P = 0.5$ by unpaired *t*-test) and were delayed, occurring ~30 s after smoke inhalation.

Time courses of the responses of the RARs and peak tracheal pressure. To determine the time courses for the mainstream smoke-evoked changes in RAR activity in the sidestream smoke- and filtered air-exposed groups and to compare them with the time courses for changes in peak tracheal pressure, we plotted RAR activity as spikes/bin on a bin-by-bin basis and the peak tracheal pressure on a breath-by-breath basis over the same time frame. The time courses for the changes in RAR activity and peak tracheal pressure after smoke from low-nicotine cigarettes are shown in Fig. 4. For the filtered air-exposed guinea pigs, the increase in RAR activity began immediately (the first 3 s) after the three breaths of mainstream smoke inhalation; the increase augmented over 12 s and then began to wane, returning to near control values within 60 s. The increase in RAR activity preceded the increase in peak tracheal pressure. Peak tracheal pressure began to increase after 8 s (4 breaths), after the three breaths of smoke inhalation, and was sustained beyond 60 s, after RAR activity had returned to near baseline values. For the sidestream smoke-exposed guinea pigs, RAR activity did not increase significantly, although peak tracheal pressure increased ~8 s (4 breaths) after smoke inhalation.

The time courses for the changes in RAR activity and peak tracheal pressure after smoke from high-nicotine cigarettes are shown in Fig. 5. For the filtered air-exposed animals, the time courses for the increase in RAR activity and the increase in peak tracheal pressure paralleled the time courses for the response to low-nicotine cigarettes. The RAR activity increased immediately after the three breaths of smoke, incremented for 9 s, and then began to decline. The increase preceded the increase in peak tracheal pressure by ~8 s (4 breaths). For the RARs from the sidestream smoke-exposed animals, the time courses for the increase in RAR activity and peak tracheal pressure were similar to those for the inhaled low-nicotine cigarette smoke in the filtered air-exposed group. Again, the increase in RAR activity in the sidestream smoke-exposed animals preceded the increase in peak tracheal pressure by ~6 s.

Responses of the SARs. The baseline activity of the

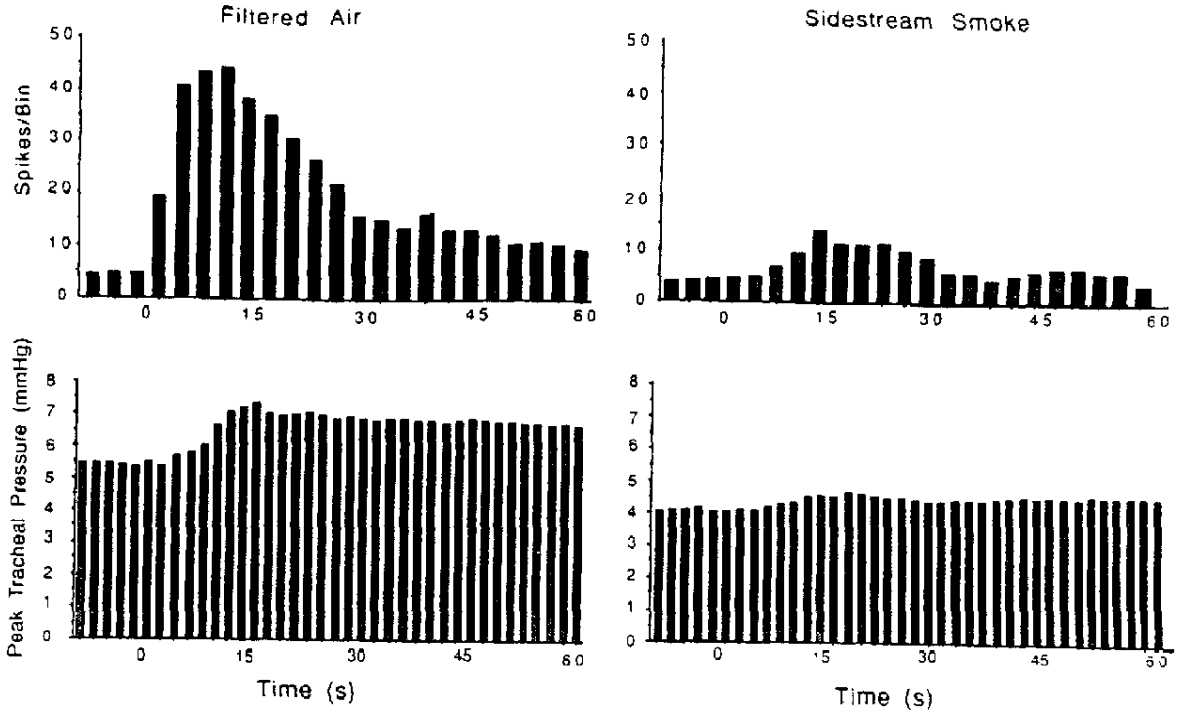

FIG. 4. Time courses for RAR and tracheal pressure responses to low-nicotine mainstream smoke in filtered air- and sidestream smoke-exposed animals. Experimental period after 3 breaths of smoke began at *time 0*. Increase in RAR activity in filtered air-exposed group preceded increase in peak tracheal pressure. Increase in peak tracheal pressure was sustained beyond 60 s, while RAR activity returned toward baseline within ~60 s. In sidestream smoke-exposed group, inhalation of low-nicotine mainstream smoke did not alter RAR activity and produced a modest increase in peak tracheal pressure that was sustained for ~60 s.

SARs was significantly higher in the sidestream smoke-exposed group, averaging 116 ± 62 spikes/bin compared with 70 ± 37 spikes/bin in the filtered air-exposed group ($P < 0.01$ by unpaired *t*-test). The SARs exhibited variable responses to smoke inhalation, and there were no differences in the percent change in responses between the filtered air- and sidestream smoke-exposed animals ($P > 0.05$ by unpaired *t*-test). In the SARs recorded from the filtered air-exposed group, six were excited, one was inhibited, and nine were not affected by inhalation of low-nicotine smoke. In the SARs recorded from the sidestream smoke-exposed animals, 4 were excited, 2 were inhibited, and 15 had no change in activity. The results were similar for the responses to smoke from high-nicotine cigarettes. In the filtered air-exposed animals, five SARs were excited, one was inhibited, and nine were unaffected. In the sidestream smoke-exposed group, nine SARs were excited, two were inhibited, and nine were unaffected. For one SAR, only low-nicotine smoke was delivered.

DISCUSSION

The principal finding of this study was that exposure to sidestream smoke in the developing guinea pig diminished the responsiveness of the RARs to inhalation of mainstream smoke from both low- and high-nicotine-containing cigarettes. Such was not the case for the SARs.

Inhalation of mainstream cigarette smoke was used to evaluate the responsiveness of the RARs in the sidestream smoke- and filtered air-exposed animals because it is a well-characterized reproducible stimulant of the RARs in a variety of species (1, 17, 18, 23, 26) and because it has some societal relevance since children raised in the homes of smokers have an increased incidence of becoming smokers themselves (21, 24, 29).

The guinea pigs used in this study were 41–45 days old. The maximum life span of the guinea pig has been reported as 7.5 yr (35). Like the human, the guinea pig shows advanced development of lung function and morphology at birth (28). Growth of male guinea pigs is not complete until 9 mo of age, at which time they weigh ~1,000 g. Their age of puberty is 5–10 wk. Thus, the developmental stage of these 41- to 45-day-old guinea pigs is similar to human childhood.

Sidestream smoke exposure had no effect on the baseline RAR activity, which averaged ~1 Hz in both the sidestream smoke- and filtered air-exposed groups. However, it markedly diminished the responsiveness of the RARs to acute inhalation of mainstream smoke. The diminution was most apparent when smoke from low-nicotine cigarettes was inhaled: the activity of the RARs from sidestream smoke-exposed guinea pigs was not significantly increased by low-nicotine mainstream

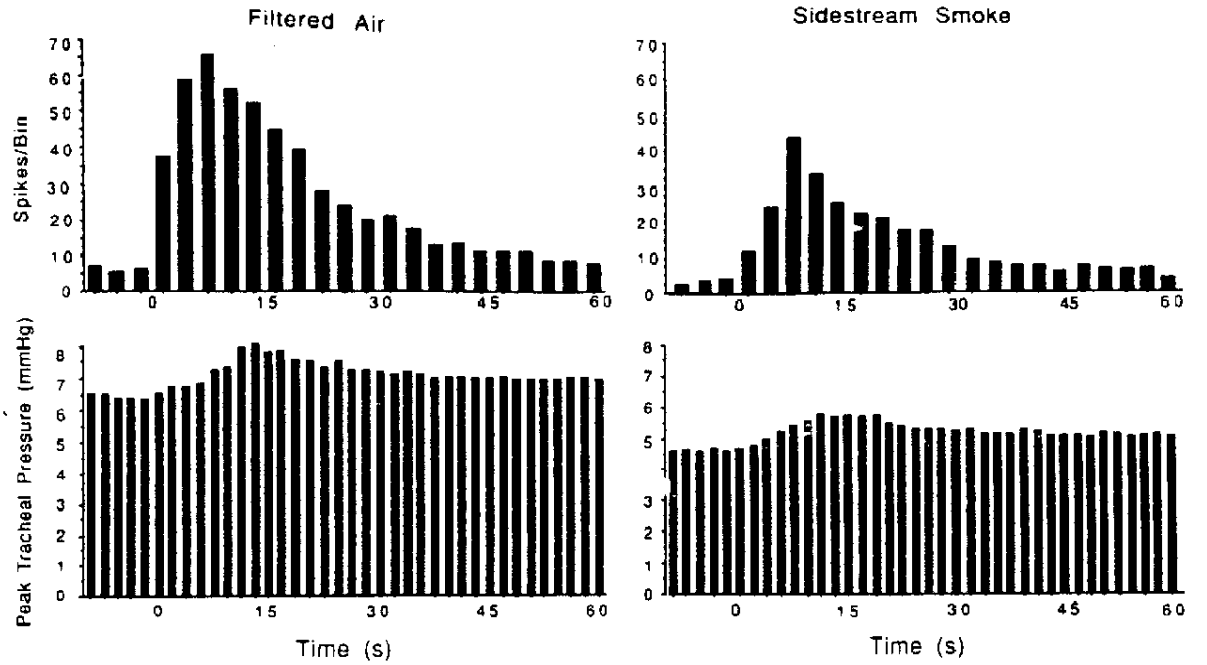

FIG. 5. Time courses for RAR and tracheal pressure responses to high-nicotine mainstream smoke in filtered air- and sidestream smoke-exposed animals. Experimental period after 3 breaths of smoke began at *time 0*. Increase in RAR activity in filtered air-exposed group preceded increase in peak tracheal pressure. Increase in peak tracheal pressure was sustained beyond 60 s, while RAR activity returned toward baseline within ~60 s. In sidestream smoke-exposed group, inhalation of low-nicotine mainstream smoke increased RAR activity and peak tracheal pressure. Increase in RAR activity in sidestream smoke-exposed group preceded increase in peak tracheal pressure. RAR activity returned toward control value after ~60 s, while increase in peak tracheal pressure was sustained for ~60 s.

smoke, whereas the activity of the RARs from the filtered air-exposed guinea pigs increased to ~10 Hz. The disparity in the responsiveness of the RARs between the two groups was still significant but less so when mainstream smoke from high nicotine-containing cigarettes was inhaled; the RAR activity in the sidestream smoke-exposed group increased to ~7 Hz, whereas that in the filtered air-exposed group increased to ~12 Hz. These observations suggest that sidestream smoke raised the threshold for responsiveness to mainstream smoke to the extent that the RARs in the sidestream smoke-exposed guinea pigs were unresponsive to the low-nicotine cigarettes and less responsive to the high-nicotine cigarettes than were the RARs in the control group.

Of related interest was the finding that the peak tracheal pressure response to mainstream smoke inhalation was qualitatively similar to the response of the RARs. Tracheal pressure was increased by mainstream smoke inhalation from low-nicotine cigarettes in both groups of guinea pigs; however, the increase was significantly greater in the filtered air-exposed group (21%) compared with the sidestream smoke-exposed group (5%). The difference in the responsiveness between the two groups was less when mainstream smoke from the high-nicotine cigarettes was inhaled. The increase in peak tracheal pressure in the filtered air-exposed group was 20% compared with a 16% increase in the sidestream smoke-exposed group. Thus, the responsiveness of the peak tracheal pressure corresponded to that of the RARs, in that the most significant difference was observed after inhaled mainstream smoke from low-nicotine cigarettes. Of note also was the finding that in the sidestream smoke-exposed guinea pigs the responses of the RARs and the peak tracheal pressure to inhalation of high-nicotine mainstream smoke were similar in both magnitude and time course to the responses evoked by the inhalation of low-nicotine smoke in the filtered air-exposed guinea pigs. Inhalation of low-nicotine mainstream smoke in the sidestream smoke-exposed guinea pigs slightly increased peak tracheal pressure, but there was only a trend toward an increase in RAR activity.

It is generally recognized that inhaled mainstream cigarette smoke may stimulate the RARs directly (17, 23, 26) or indirectly by contracting airway smooth muscle (1, 25, 37). Furthermore, mainstream cigarette smoke may contract airway smooth muscle by activation of cholinergic ganglia in the airways (12), by a central reflex initiated by RAR activation (15), by a central (5, 18) or local axon reflex (15, 19) initiated by the pulmonary or bronchial C-fiber receptors, or by stimulation of the central chemoreceptors (10, 11). In the present study, the guinea pigs were artificially ventilated and peak tracheal pressure was used as a global index of a change in bronchomotor tone and/or lung

compliance. Even though RAR activity is sensitive to both increases in airway tone and decreases in lung compliance (14), examination of the time courses for the responses of the RARs and the peak tracheal pressure after mainstream smoke inhalation suggested that *1*) the increase in RAR activity preceded the increase in bronchomotor tone and/or the decrease in lung compliance and 2) the RAR activity returned to the control value despite a sustained increase in peak tracheal pressure. These observations argue that some constituent of the mainstream smoke, rather than an increase in airway tone or decrease in lung compliance, was the stimulus for the RARs. It seems more likely that stimulation of the RARs may have contributed to the increase in peak tracheal pressure.

Although not the main focus of this study, the finding that the baseline peak tracheal pressure was slightly lower (19%) in the sidestream smoke-exposed guinea pigs was of interest. The data may be explained by the previous findings of Joad et al. (13) that sidestream smoke exposure in developing guinea pigs produced modest but significant (17%) increase in dynamic lung compliance with no change in lung resistance.

Of consideration, also, is whether sidestream smoke exposure diminishes the responsiveness of the RARs specifically to mainstream smoke or generally to other stimulants. The diminished responsiveness to mainstream smoke may contribute to the increased incidence of children who are raised with smokers becoming smokers themselves (21, 24, 29). That is, the immediate discomfort of the airway irritation and cough that accompanies the initial smoking experience, which may be a deterrent for some potential smokers, is less in those individuals raised with ETS. If the decreased responsiveness of the RARs is a general phenomenon, the question arises as to whether it is harmful or helpful. If the bronchoconstriction and mucus secretion elicited by the RARs as well as by the pulmonary and bronchial C-fiber receptors are, indeed, part of a defensive reflexive response that protects the airways from inhalation of noxious substances, then a diminution of the RAR responsiveness to mainstream smoke may weaken the reflex response, making the lungs and airways more vulnerable. On the other hand, Coleridge and Coleridge (4) have recently conjectured that, ontologically, the reflexive responses may have lost their defensive function and, in fact, may contribute to the discomfort evoked by stimulation of the vagal sensory receptors. It seems reasonable to expect, however, that even if the defensive function is lost, the immediate airway discomfort evoked by the initial smoking experience might still have a deterrent function.

In conclusion, exposing guinea pigs to sidestream tobacco smoke during their development diminishes the responsiveness of RARs to acute inhalation of mainstream smoke. If this is true for humans, the most direct implication may be that the potential deterrent effect of the initial uncomfortable response to smoking is lessened in children raised with ETS. There may be a broader implication if the diminished responsiveness of the RARs is generalized to other irritants. That is, the lung may become more vulnerable to other noxious agents, which may explain, at least in part, the increased incidence of respiratory symptoms in children raised in the homes of smokers.

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